

Focus: Metabolism

Hypoxia-inducible factors: coupling glucose metabolism and redox regulation with induction of the breast cancer stem cell phenotype

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Abstract

Reduced oxygen availability (hypoxia) leads to increased production of reactive oxygen species (ROS) by the electron transport chain. Here, I review recent work delineating mechanisms by which hypoxia-inducible factor 1 (HIF-1) mediates adaptive metabolic responses to hypoxia, including increased flux through the glycolytic pathway and decreased flux through the tricarboxylic acid cycle, in order to decrease mitochondrial ROS production. HIF-1 also mediates increased flux through the serine synthesis pathway and mitochondrial one-carbon (folate cycle) metabolism to increase mitochondrial antioxidant production (NADPH and glutathione). Dynamic maintenance of ROS homeostasis is required for induction of the breast cancer stem cell phenotype in response to hypoxia or cytotoxic chemotherapy. Consistently, inhibition of phosphoglycerate dehydrogenase, the first enzyme of the serine synthesis pathway, in breast cancer cells impairs tumor initiation, metastasis, and response to cytotoxic chemotherapy. I discuss how these findings have important implications for understanding the logic of the tumor microenvironment and for improving therapeutic responses in women with breast cancer.

Keywords cancer; one-carbon metabolism; pluripotency; progression; serine synthesis

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Introduction

Over the last decade, a paradigm shift has occurred in our understanding of the role of glycolysis in glucose metabolism. It was previously thought that hypoxic cells utilize glycolysis because it provides a means of producing ATP when O₂ is not available for respiration. However, cells switch from oxidative to glycolytic metabolism long before O₂ becomes limiting for oxidative phosphorylation and a major driving force behind this metabolic switch is not just to maintain production of ATP but also to prevent excessive

production of reactive oxygen species (ROS). Because sustained increases in ROS levels lead to cellular dysfunction and death, glucose metabolism is reprogrammed in hypoxic breast cancer cells in order to maintain redox homeostasis and cell survival. This short review will summarize the molecular and physiological basis for hypoxia-induced metabolic reprogramming of breast cancer cells, with a particular focus on breast cancer stem cells (BCSCs), which are a small subpopulation of cells within a tumor that are capable of self-renewal and unlimited replication, and are the only cells capable of forming the secondary (recurrent and metastatic) tumors that are responsible for breast cancer mortality (Al-Hajj *et al*, 2003; Charafe-Jauffret *et al*, 2009). The review will focus on the effects of intratumoral hypoxia on glucose metabolism in breast cancer and will not discuss O₂-independent effects of somatic mutations on metabolism (the so-called Warburg effect).

Glucose metabolism and ROS

Glucose is converted to pyruvate in the Embden–Meyerhof (glycolytic) pathway (EMP), followed by conversion of pyruvate to acetyl-CoA and its oxidation in the tricarboxylic acid (TCA) cycle with the generation of reducing equivalents (NADH and FADH₂) that are utilized by the electron transport chain (ETC), which generates an electrochemical gradient of H⁺ ions across the inner mitochondrial membrane that is used to drive the synthesis of ATP, with O₂ serving as the ultimate electron acceptor (Fig 1). Alternatively, lactate dehydrogenase A (LDHA) converts pyruvate to lactate as the terminal product of glycolysis.

Although oxidative metabolism is critical for the generation of sufficient energy to support the development and maintenance of complex multicellular organisms (Kump, 2010; Lane & Martin, 2010), exposure of cells to excess O₂ leads to the generation of ROS, such as superoxide anions, as a result of electrons reacting with O₂ prior to complex IV (Fig 1). ROS have the potential to oxidize cellular macromolecules and, by doing so, cause cell dysfunction or death (Lane, 2011). Studies performed with isolated mitochondria suggested a linear relationship between O₂ concentration and superoxide production (Turrens *et al*, 1982). However, acute exposure of

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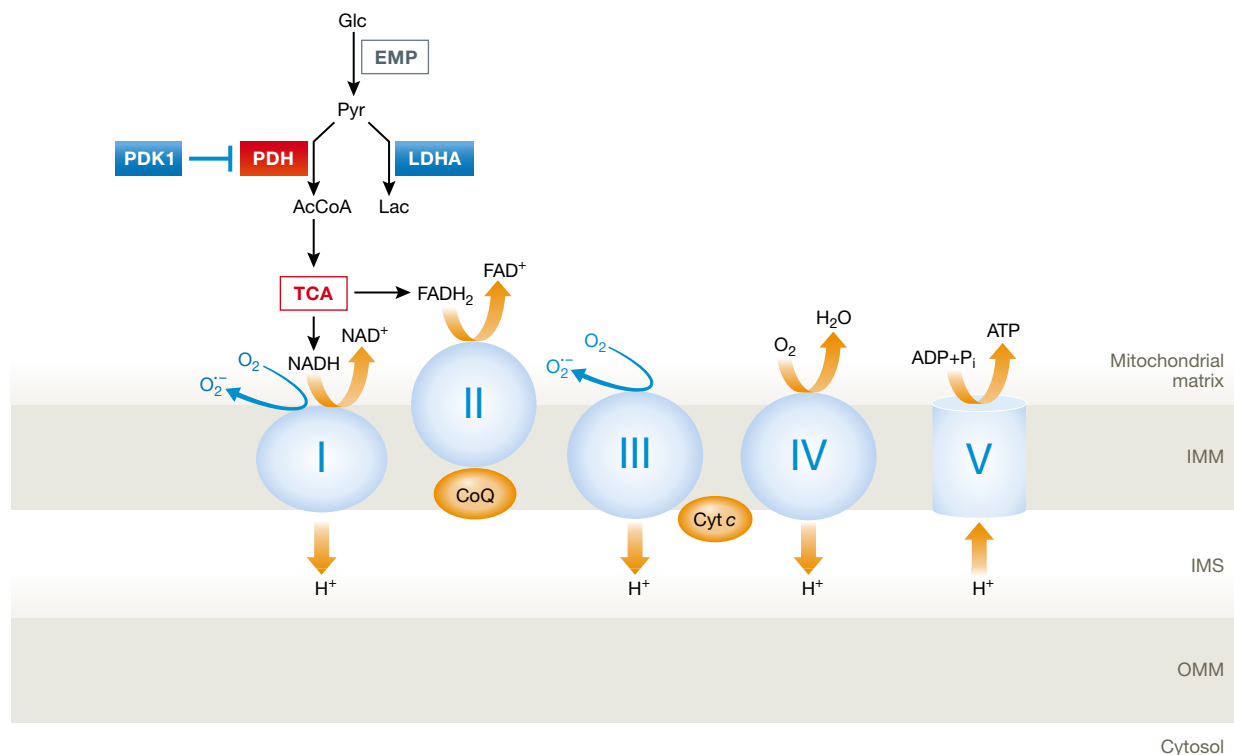


Figure 1. Glycolytic and oxidative metabolism of glucose.

In well-oxygenated cells, the Embden–Meyerhof pathway (EMP) reactions convert glucose (Glc) to pyruvate (Pyr), which is converted to acetyl-CoA (AcCoA) by pyruvate dehydrogenase (PDH) for entry into the tricarboxylic acid (TCA) cycle, in which AcCoA is oxidized to $\text{CO}_2 + \text{H}_2\text{O}$ and reducing equivalents are generated (NADH and FADH_2) for donation to the electron transport chain (complex I and complex II, respectively). Proton (H^+) pumping during electron transport generates an electrochemical gradient that is used to synthesize ATP (complex V) with O_2 serving as the ultimate electron acceptor (complex IV). Premature transfer of electrons to O_2 (at complex I or complex III) results in the formation of superoxide anion. Under hypoxic conditions, superoxide production at complex III increases, leading to the hypoxia-inducible factor 1 activation and increased expression of lactate dehydrogenase A (LDHA), which converts pyruvate to lactate (Lac), and PDH kinase 1 (PDK1), which inhibits the conversion of pyruvate to AcCoA, leading to decreased flux through the TCA cycle and decreased mitochondrial ROS production. CoQ, coenzyme Q (ubiquinone); Cyt c, cytochrome c; IMM, inner mitochondrial membrane; IMS, intermembrane space; OMM, outer mitochondrial membrane.

mammalian cells to hypoxic conditions (1% O_2 , $\text{PO}_2 = 7$ mmHg) leads to increased ROS generation at ETC complex III (Guzy *et al*, 2005). Thus, both hyperoxia and hypoxia increase mitochondrial ROS generation, indicating that the ETC functions most efficiently under normoxic (i.e. physiological) conditions, which for most mammalian cells is in the PO_2 range of 20–65 mmHg (3–9% O_2), although PO_2 in the bone marrow is as low as 10 mmHg (1.4%) (Spencer *et al*, 2014). In tumors, oxygenation is severely reduced compared to normal tissue, with an overall median PO_2 of 10 mmHg in head and neck, cervical, and breast cancers and an overall hypoxic fraction ($\text{PO}_2 \leq 2.5$ mmHg) of ~25% (Vaupeul *et al*, 2007).

Although conventional wisdom held that glycolysis represented a default pathway for ATP production when O_2 was not available, cells actively repress expression of genes encoding respiratory chain components and induce expression of genes encoding glycolytic enzymes at 2% O_2 ($\text{PO}_2 = 14$ mmHg), which is not limiting for respiration (Webster, 1987; Webster *et al*, 1990). The coordinate transcriptional induction of genes encoding glycolytic enzymes in response to hypoxia is mediated by hypoxia-inducible factor 1 (HIF-1; Semenza *et al*, 1994, 1996; Iyer *et al*, 1998). In addition, HIF-1 was shown to activate transcription of the *PDK1* gene encoding pyruvate dehydrogenase kinase 1, which phosphorylates and inactivates pyruvate dehydrogenase (PDH). Pyruvate can be

converted to acetyl-CoA by PDH or to lactate by LDHA (Fig 1). HIF-1 activates expression of both *PDK1* and *LDHA* to switch cells from oxidative to glycolytic metabolism (Semenza *et al*, 1996; Iyer *et al*, 1998; Kim *et al*, 2006; Papandreou *et al*, 2006).

The importance of this metabolic switch was paradigm shifting: HIF-1 α -knockout mouse embryo fibroblasts, which were unable to switch to glycolytic metabolism in response to hypoxia, had higher ATP levels than wild-type cells (i.e. 1% O_2 was not limiting for oxidative phosphorylation), but the cells died due to overwhelming levels of ROS and could be rescued by forced expression of PDK1 or by treatment with ROS scavengers (Kim *et al*, 2006; Zhang *et al*, 2008). Subsequent studies identified additional mechanisms by which HIF-1 can suppress oxidative metabolism, including inhibition of fatty acid oxidation, which also generates acetyl-CoA (Huang *et al*, 2014), induction of mitochondrial-selective autophagy (Zhang *et al*, 2008; Bellot *et al*, 2009), and inhibition of ETC complex I activity (Chan *et al*, 2009; Favaro *et al*, 2010; Tello *et al*, 2011).

Taken together, the studies described above demonstrate that HIF-1 dynamically regulates glucose metabolism based on O_2 availability to defend against the risk of sustained increased ROS production. It should be stated explicitly that hypoxic cells do not metabolize all of their glucose to lactate, nor do non-hypoxic cells oxidize all of their glucose to carbon dioxide and water. The

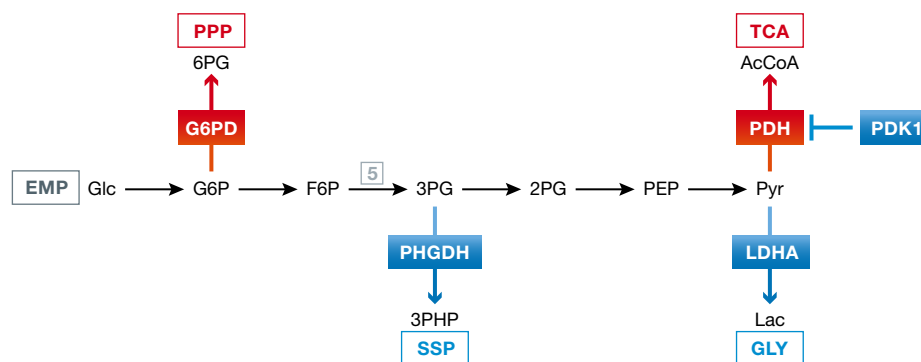


Figure 2. Glucose metabolic shunt pathways that generate NADPH.

The first reaction of the EMP converts glucose (Glc) to glucose-6-phosphate (G6P), which is either converted to fructose-6-phosphate (F6P) or shunted to the pentose phosphate pathway (PPP) by glucose-6-phosphate dehydrogenase (G6PD), which converts G6P to 6-phosphogluconate (6PG). Five steps later in the EMP, 3-phosphoglycerate (3PG) is either converted to 2-phosphoglycerate (2PG) or shunted to the serine synthesis pathway (SSP) by phosphoglycerate dehydrogenase (PHGDH), which converts 3PG to 3-phosphohydroxypyruvate (3PHP). The PPP generates cytosolic NADPH, whereas the SSP can lead to the generation of either cytosolic or mitochondrial NADPH. Cellular O_2 availability determines flux through these pathways in breast cancer cells: Hypoxia represses G6PD expression and induces the expression of PHGDH, LDHA, and PDK1. PEP, phospho-enol-pyruvate; Pyr, pyruvate; Lac, lactate; GLY, glycolytic end-product.

metabolic set point (i.e. the balance between oxidative and reductive metabolism) varies from one cell type to another, as does the extent to which the balance shifts away from oxidative metabolism under hypoxic conditions. Furthermore, as discussed in detail below, redox homeostasis is dependent upon the balance between oxidant and antioxidant production.

NADPH production

Two shunt pathways, the pentose phosphate pathway (PPP) and serine synthesis pathway (SSP), divert glucose metabolites (Fig 2), which was believed to be important for the production of substrates for lipid and nucleotide synthesis. However, these are the major pathways that generate NADPH, which is used to maintain glutathione, the principal cellular antioxidant, in a reduced state. The PPP generates NADPH in the cytosol, whereas serine generated by the SSP is used as a substrate for one-carbon (folate cycle) metabolism (1CM), which generates NADPH, in either the cytosol or mitochondria. Historically, these pathways have been considered

mostly in the context of cell proliferation (Jain *et al*, 2012), rather than redox homeostasis, and NADPH is often not even included on pathway maps. However, recent studies have demonstrated that 1CM is the major contributor to cellular NADPH production (Fan *et al*, 2014).

In the SSP (Fig 3), phosphoglycerate dehydrogenase (PHGDH) converts the glucose metabolite 3-phosphoglycerate (3PG) to 3-phosphohydroxypyruvate (3PHP); phosphoserine aminotransferase 1 (PSAT1) converts 3PHP to phosphoserine (PSer); and phosphoserine phosphatase (PSPH) converts PSer to serine (Fig 3, left). In mitochondrial 1CM (mito1CM), serine hydroxymethyltransferase 2 (SHMT2) catalyzes the reaction of serine and tetrahydrofolate (THF) to form glycine and methylene tetrahydrofolate (MTHF); next, MTHF dehydrogenase 2 (MTHFD2) catalyzes the reaction of MTHF and $NADP^+$ to form formyl-THF and NADPH; and, finally, MTHFD1L splits formyl-THF into formate and THF (Fig 3, right). In cytosolic 1CM (cyto1CM), the same reactions are carried out by SHMT1 and MTHFD1, which catalyzes the reactions performed by both MTHFD2 and MTHFD1L (Tibbets & Appling, 2010).

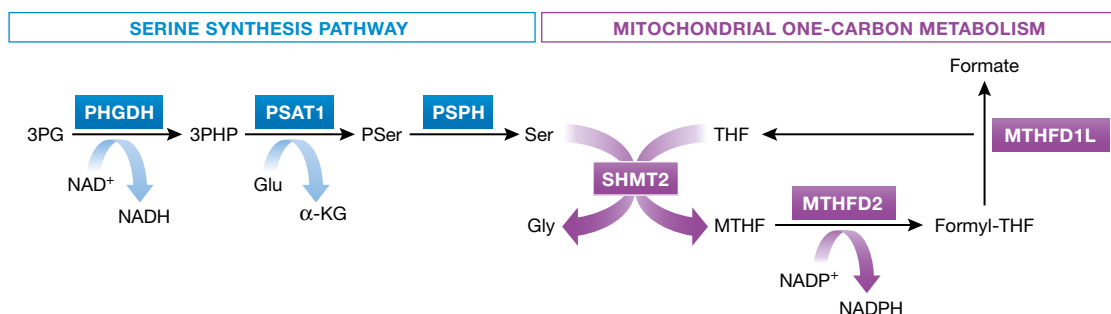


Figure 3. Mitochondrial one-carbon (folate cycle) metabolism generates mitochondrial NADPH.

The serine synthesis pathway converts 3PG to serine (Ser) through the activity of PHGDH, phosphoserine aminotransferase 1 (PSAT1), and phosphoserine phosphatase (PSPH). Ser is a substrate for mitochondrial one-carbon metabolism, which generates NADPH while converting Ser + tetrahydrofolate (THF) into glycine (Gly) + THF + formate through the activity of serine hydroxymethyl transferase 2 (SHMT2), methylene-THF dehydrogenase (MTHFD) 2, and MTHFD1L. Glu, glutamate; α -KG, α -ketoglutarate.

Given that HIF-1 mediates a switch from oxidative to glycolytic metabolism leading to decreased production of mitochondrial oxidants, we hypothesized that it might also mediate increased production of mitochondrial antioxidants. Indeed, hypoxia coordinately induced the expression of the mRNAs encoding all three SSP (PHGDH, PSAT1, PSPH) and all three mito1CM (SHMT2, MTHFD2, MTHFD1L) enzymes in four out of six human breast cancer cell lines analyzed, with PHGDH and SHMT2 expression induced by hypoxia in all six lines, which included examples of both estrogen receptor-positive (ER⁺) and ER⁻ breast cancer (Samanta *et al*, 2016). Coordinate, HIF-mediated transcriptional induction of genes encoding SSP and mito1CM enzymes, as previously demonstrated for the EMP (Iyer *et al*, 1998; Seagroves *et al*, 2001), ensures increased flux through the pathway. In contrast, expression of genes encoding cyto1CM enzymes was not consistently induced by hypoxia. Expression of G6PD, which encodes the first enzyme of the PPP (Fig 2), was repressed by hypoxia in all breast cancer cell lines analyzed, and levels of 6-phosphogluconate, the product of the G6PD reaction, were significantly decreased in response to hypoxia (Samanta *et al*, 2016). These results suggest that breast cancer cells shift from cytosolic to mitochondrial NADPH production under hypoxic conditions.

Knockdown of PHGDH expression in ER⁺ or ER⁻ breast cancer cell lines increased both respiration and glycolysis and indicated that one-quarter to one-half of all glucose metabolites were shunted to the SSP in control cells. Loss of PHGDH expression led to decreased NADPH levels, decreased ratio of reduced:oxidized glutathione, increased mitochondrial superoxide levels, and increased apoptosis under hypoxic conditions (Samanta *et al*, 2016). These results confirmed our hypothesis that HIF-1 mediates increased antioxidant production in hypoxic breast cancer cells.

Breast cancer stem cells

Hypoxia increases the percentage of breast cancer stem cells (BCSCs) in a HIF-1-dependent manner (Conley *et al*, 2012; Xiang *et al*, 2014). HIF-1 also plays a critical role in maintaining cancer stem cells under hypoxic conditions in glioblastoma (Qiang *et al*, 2012). We previously proposed that stem cell metabolism was designed to protect the cells against oxidant exposure (Suda *et al*, 2011). Exposure of ER⁺ MCF-7 cells or ER⁻ MDA-MB-231 cells to 1% O₂ for 3 days induced a fivefold increase in the percentage of BCSCs, which was abrogated by PHGDH knockdown (Samanta *et al*, 2016). PHGDH knockdown in MDA-MB-231 cells did not affect the efficiency of tumor formation when 2 × 10⁶ breast cancer cells were injected into the mammary fat pad of immunodeficient mice, such that BCSCs were not limiting; however, the percentage of BCSCs in the resulting tumors was reduced ~4-fold. Under conditions in which BCSCs were limiting (injection of only 1 × 10³ cells), tumors formed in only six out of 14 mice injected with PHGDH-knockdown breast cancer cells as compared to seven out of seven mice injected with control cells. Although primary tumor growth was not inhibited by PHGDH knockdown, spontaneous metastasis from breast to lungs was dramatically impaired, as predicted by the reduced number of BCSCs within PHGDH-knockdown primary tumors (Samanta *et al*, 2016). These results complement an earlier study, which reported that an MDA-MB-231 subclone with increased

bone metastasis overexpressed PHGDH, PSAT1, and PSPH (Pollari *et al*, 2011), although the authors of that study speculated on the potential role of serine in osteoclast, rather than BCSC, biology.

PHGDH knockdown does not impair proliferation of most breast cancers

The *PHGDH* gene is amplified in ~6% of human breast cancers, and proliferation of breast cancer cell lines with *PHGDH* amplification is impaired by PHGDH knockdown (Locasale *et al*, 2011; Possemato *et al*, 2011). In contrast, loss of PHGDH expression does not seem to impair growth of cells without *PHGDH* amplification, as PHGDH knockdown (using any one of five different shRNAs) had no effect on the proliferation of ER⁺ MCF-7 cells *in vitro* and actually increased the growth of ER⁻ MDA-MB-231 cells, both *in vitro* and *in vivo* (Samanta *et al*, 2016). Our results suggest that the SSP does not play an important role in “biomass accumulation” in the majority of breast cancers that do not have *PHGDH* amplification.

Related findings in other types of cancer

In patient-derived melanoma xenografts, cytosolic ROS levels were significantly higher in cells that were either circulating in the blood or located within metastatic nodules, as compared to melanoma cells in subcutaneous tumors, whereas mitochondrial ROS levels were significantly elevated only in metastases, which had increased levels of NADP⁺ and NADPH (Piskounova *et al*, 2015). The authors implicated ALDH1L2 and MTHFD1, which, like MTHFD2, are NADPH-generating enzymes, in the response to oxidative stress in metastatic melanoma cells (Piskounova *et al*, 2015). In non-small-cell lung carcinoma, the transcription factors ATF4 and NRF2 were found to positively regulate the expression of the *PHGDH*, *PSAT1*, and *SHMT2* genes (DeNicola *et al*, 2015). In hepatocellular carcinoma, C-MYC-dependent induction of SSP activity under conditions of nutrient deprivation was reported and PSPH expression was associated with patient mortality (Sun *et al*, 2015). In *N-MYC*-amplified neuroblastoma cells, hypoxia induced SHMT2 expression in a HIF-1-dependent manner, SHMT2 knockdown led to increased ROS and cell death under hypoxic conditions (similar to the effect of PHGDH knockdown in breast cancer cells), and immunohistochemistry revealed a significant correlation between HIF-1α and SHMT2 expression in neuroblastoma sections (Ye *et al*, 2014). In glioblastoma, SHMT2 was found to promote survival of ischemic cancer cells, but the role of ROS was not investigated (Kim *et al*, 2015). Further studies are needed to determine the role of ATF4, C-MYC, N-MYC, and NRF2 in regulating the SSP and 1CM in breast cancer cells and the role of HIF-dependent reprogramming of the SSP and 1CM in neoplasms other than breast cancer.

Cancer therapy induces HIF-1 activity

HIF-1 has been implicated in chemotherapy resistance (Unruh *et al*, 2003), but recent studies have demonstrated that exposure of breast cancer cells to cytotoxic chemotherapy induces HIF-1 activity and increases the percentage of BCSCs in a HIF-dependent manner (Cao

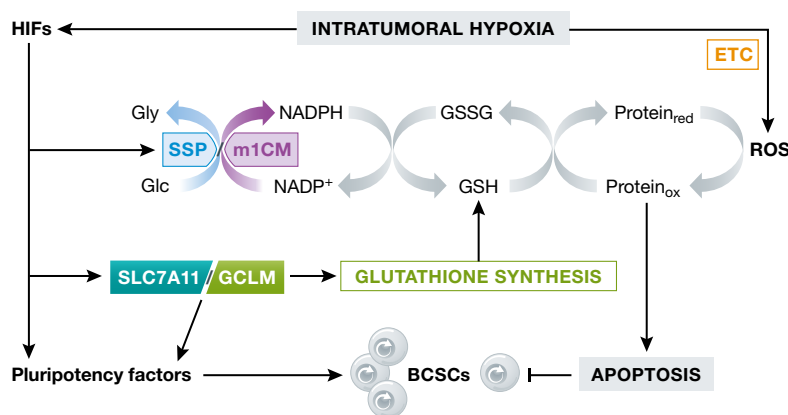


Figure 4. HIFs increase antioxidant production to maintain redox homeostasis, which is required for induction of the breast cancer stem cell phenotype under hypoxic conditions.

Under conditions of acute hypoxia, the production of reactive oxygen species (ROS) by the electron transport chain (ETC) is increased. HIFs activate the transcription of the following: genes encoding enzymes of the serine synthesis pathway (SSP) and mitochondrial one-carbon metabolism (m1CM) to increase the production of NADPH, which is used to convert oxidized glutathione (GSSG) to its reduced form (GSH); and genes encoding SLC7A11 and GCLM to increase glutathione production. Reduced glutathione is used to reverse the oxidation of cellular proteins (Protein_{ox}) to their reduced forms (Protein_{red}). HIFs also induce expression of pluripotency factors that specify the breast cancer stem cell (BCSC) phenotype. Hypoxia induces the BCSC phenotype, but only if mitochondrial redox homeostasis is maintained.

et al, 2013; Samanta *et al*, 2014; Lu *et al*, 2015). Compared to control breast cancer cells, exposure of PHGDH-knockdown subclones to carboplatin or doxorubicin did not lead to an enrichment of BCSCs but resulted in significantly increased mitochondrial ROS levels and apoptosis, both *in vitro* and *in vivo* (Samanta *et al*, 2016).

Anti-angiogenic therapy blocks VEGF signaling, inhibits tumor vascularization, and decreases tumor growth but increases tumor hypoxia, leading to induction of HIF-1 activity, which promotes cancer progression (Ebos & Kerbel, 2011; Semenza, 2016). However, combination therapy with a HIF-1 inhibitor (topotecan or CRLX101, a nanoparticle drug conjugate containing camptothecin) prevented the induction of HIF-1 by bevacizumab and increased survival in a mouse model of metastatic breast cancer (Rapisarda *et al*, 2009; Pham *et al*, 2016).

Are these findings relevant to women with breast cancer? Analysis of gene expression data from 1,215 human breast cancers revealed a highly significant correlation between levels of PHGDH, PSAT1, PSPH, SHMT2, MTHFD2, and MTHFD1L mRNA (either individually or in aggregate) and the expression of a HIF signature consisting of mRNAs encoding HIF-1 α and 13 HIF target genes. Analysis of over 3,500 breast cancers revealed that patient mortality was significantly increased when expression of PHGDH mRNA ($P < 10^{-6}$) or mRNAs encoding all six SSP and mito1CM enzymes ($P < 10^{-16}$) was greater than the median level (Samanta *et al*, 2016).

The studies described above indicate that exposure of breast cancer cells to hypoxia induces a reallocation of glucose metabolites away from the PPP and toward the SSP. In contrast, treatment of SUM159 triple-negative breast cancer cells with a histone deacetylase (HDAC) inhibitor (valproic acid or suberoylanilide hydroxamic acid) for 7 days increased the percentage of BCSCs and levels of total cellular ROS, mitochondrial ROS, NADPH, and G6PD mRNA (Debeb *et al*, 2016). However, the effect of HDAC inhibitors on glucose metabolites or SSP/1CM enzyme expression was not investigated, nor was the effect of G6PD knockdown on BCSC enrichment

determined. Thus, further studies are required in order to interpret these interesting findings.

Hypoxia induces glutathione synthesis

In breast cancer cells, hypoxia also induces the HIF-dependent expression of *SLC7A11* and *GCLM*, which are direct HIF target genes (as determined by chromatin immunoprecipitation assays) and encode components of the glutathione biosynthetic pathway (Lu *et al*, 2015). Glutathione (γ -L-glutamyl-L-cysteinylglycine) is a tripeptide generated from cysteine (transported into the cell by the *SLC7A11* gene product), which is joined to glutamate, in a reaction catalyzed by glutamate-cysteine ligase (the regulatory subunit of which is encoded by *GCLM*), and then to glycine (the product of the SHMT reaction). Hypoxia-induced glutathione synthesis complements the increased NADPH synthesis that is mediated by induction of the SSP and mito1CM genes (Fig 4). As in the case of PHGDH, knockdown of either *SLC7A11* or *GCLM* expression in human breast cancer cells impairs tumor initiation and abrogates chemotherapy-induced enrichment of BCSCs (Lu *et al*, 2015). Breast cancer initiation is also impaired in *Gclm*^{-/-} mice (Harris *et al*, 2015). Thus, HIF-1 functions as a master regulator of mitochondrial ROS homeostasis and the BCSC phenotype by mediating increased mitochondrial antioxidant production under hypoxic conditions.

Do HIFs promote BCSC specification or maintenance?

This review has focused on the role of HIFs in maintaining redox homeostasis under hypoxic conditions, which is required for the induction of the BCSC phenotype. In the absence of redox homeostasis, it is likely that BCSCs are highly susceptible to ROS-induced cell death. However, it should be acknowledged that PHGDH knockdown was shown to increase apoptosis of the bulk cancer cell

population under hypoxic conditions; a specific effect of ROS on the survival of BCSCs has not been formally demonstrated. Nevertheless, it is likely that HIFs play a role in BCSC maintenance by promoting their survival.

However, it is clear that HIFs also contribute to BCSC specification by increasing the expression of genes encoding pluripotency factors. One mechanism is quite indirect: HIF-1-dependent *SLC7A11* and *GCLM* expression leads to increased production of glutathione, which chelates copper, thereby inactivating MEK-ERK signaling, which would otherwise result in the cytoplasmic sequestration of FOXO3, a transcription factor that directly activates transcription of *NANOG*, which encodes a pluripotency factor that specifies the BCSC phenotype (Lu *et al*, 2015). In hypoxic breast cancer cells, HIFs also activate transcription of the *ZNF217* and *ALKBH5* genes, which encode proteins that inhibit methylation or mediate demethylation, respectively, of *NANOG* mRNA, thereby increasing its half-life and increasing *NANOG* protein levels (Zhang *et al*, 2016a,b). Finally, HIFs may directly transactivate genes encoding pluripotency factors under hypoxic conditions (Mathieu *et al*, 2011).

Implications for novel therapeutic approaches

Breast cancers that express the progesterone receptor (PR) and/or ER are treated with aromatase inhibitors or tamoxifen and HER2-positive breast cancers are treated with trastuzumab, but there are no targeted therapies available for triple-negative (PR⁻/ER⁻/HER2⁻) breast cancers, which are treated with cytotoxic chemotherapy. Because BCSCs are responsible for recurrent and metastatic tumors (Oskarsson *et al*, 2014; Brooks *et al*, 2015), the HIF-dependent enrichment of BCSCs by cytotoxic chemotherapy (Samanta *et al*, 2014; Lu *et al*, 2015) provides a potential explanation for the observation that treatment of triple-negative breast cancer with chemotherapy often results in a complete response that is followed by early disease recurrence, metastasis, and patient mortality (Foulkes *et al*, 2010).

Our studies suggest that combining chemotherapy with an inhibitor of the antioxidant metabolic pathways discussed above may improve patient survival. Small-molecule inhibitors of PHGDH catalytic activity have been reported recently (Mullarky *et al*, 2016; Pacold *et al*, 2016) and may be useful in the ~6% of human breast cancers that have *PHGDH* amplification (Locasale *et al*, 2011; Possemato *et al*, 2011). A recent study reported that the *PSPH* gene is also subject to amplification in breast cancer (Haider *et al*, 2016). The mito1CM pathway may also be a good target, as MTHFD2 is the metabolic enzyme that was most frequently overexpressed across all human cancers analyzed (Nilsson *et al*, 2014). Alternatively, HIF inhibitors would target a greater number of metabolic pathway components as well as genes that promote many other aspects of breast cancer progression (Semenza, 2016). Gemcitabine treatment of mice bearing MDA-MB-231 orthotopic triple-negative breast tumors controlled tumor growth but increased the percentage of BCSCs in the residual tumor and rapid tumor regrowth resumed as soon as therapy was discontinued, whereas combination therapy with the HIF-1 inhibitor digoxin prevented BCSC enrichment and resulted in tumor eradication (Samanta *et al*, 2014).

Perspective

Solid tumors contain oxygen gradients, with PO_2 decreasing as distance from the nearest blood vessel increases. Oxygen has profound and dynamic effects on the phenotype of cancer cells, altering their metabolism and replicative potential. Hypoxic cells furthest from blood vessels utilize non-oxidative metabolic pathways that enable them to maintain redox homeostasis. These cells divide relatively slowly but express pluripotency factors that endow them with a capability for self-renewal and infinite replicative potential. However, if these cancer cells migrate a few hundred μ m to a perivascular location, the increased O_2 levels extinguish HIF-dependent gene expression, leading to a switch to oxidative metabolism, which enables these cells to utilize lactate generated by hypoxic cells as an energy substrate. Loss of HIF activity also switches off the cancer stem cell program, and the cells are licensed to undergo rapid proliferation for a finite number of cell cycles. There are three paradigm-shifting conclusions that can be drawn from this view of cancer biology: First, cancer stem cells do not represent a fixed stage in a unidirectional differentiation hierarchy but rather a metabolic/physiological state that is based on micro-environmental conditions; second, there is a selective advantage associated with the formation of intratumoral O_2 gradients, which enable functional specialization, in terms of both metabolism and proliferation; and third, to counter that specialization will in many cases require the combined administration of two classes of anti-cancer drugs: those that target oxygenated cells and those that target hypoxic cells. The good news is that in many tumors, cytotoxic chemotherapy targets oxygenated, proliferating cells. It is ironic that the hypoxic cancer cells—those with the greatest propensity for establishing the lethal metastatic phenotype—would be the last to capture the attention of oncologists. Although there is increasing attention to the concept of personalized medicine based on tumor genotype, it appears likely that effective therapy will depend on targeting cancer based on metabolic phenotype as well as genotype.

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Conflict of interest

The authors declare that they have no conflict of interest.

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